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(54) Title: HO-1 SUPPRESSOR AS A DIAGNOSTIC AND PROGNOSTIC TEST FOR DEMENTING DISEASES
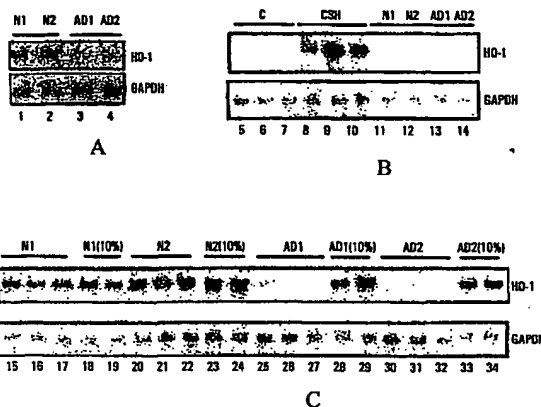
(57) Abstract: The invention relates to an improved method for predicting the onset of, diagnosing, prognosticating and/or treating dementing diseases. The method comprises determining the level of heme oxygenase-1 suppressor (HOS) activity and/or factor in tissue or body fluid obtained from a patient, and comparing said level with the corresponding level of HOS activity and/or factor in corresponding tissue or body fluid obtained from at least one control person. The tissue or body fluid is suitably blood, plasma, lymphocytes, cerebrospinal fluid, urine, saliva, epithelia or fibroblasts. The method is useful where the dementing disease is any of Alzheimer Disease, Age-Associated Cognitive Decline, Mild Cognitive Impairment, Parkinson disease with dementia, Progressive Supranuclear Palsy, Vascular (i.e. multi-infarct) Dementia, Lewy Body Dementia, Huntington's Disease, Down's syndrome, normal pressure hydrocephalus, corticobasal ganglionic degeneration, multisystem atrophy, head trauma, neurosyphilis, Creutzfeld-Jacob disease and other prion diseases, HIV and other encephalitides, and metabolic disorders such as hypothyroidism and vitamin B12 deficiency. The method may also prove useful in differentiating the "pseudodementia" of depression from Alzheimer disease.

HO-1 SUPPRESSOR AS A DIAGNOSTIC AND PROGNOSTIC TEST FOR DEMENTING DISEASES

FIELD OF THE INVENTION

Applicant's related US Patent No. 6,210,895 (April 3,
2001), herein incorporated by reference, relates to a method
for predicting, diagnosing, and/or prognosticating dementing
diseases such as Alzheimer Disease (AD) and Age-Associated
Cognitive Decline (AACD). The invention relates to improved
methods for predicting, diagnosing, prognosticating and/or
treating dementing diseases such as Alzheimer Disease (AD) and
Age-Associated Cognitive Decline (AACD) or Mild Cognitive
Impairment (MCI) as well as methods and reagents to facilitate
the study of the cause and progression of these diseases.

BACKGROUND OF THE INVENTION

Alzheimer Disease (AD) is a neurodegenerative disease
which causes dementia. The terms "Alzheimer Disease" and
"Alzheimer's Disease" are both utilized in the art, these terms
being equivalent and are used interchangeably here and
elsewhere. The period from first detection of AD to
termination can range from a few years to 15 years, during
which time the patient progressively suffers loss of both
mental function and control of bodily functions. There is
significant variability in the progress of the disease. While
the majority of patients have a gradual, inexorable progression
(losing on average 3 to 4 points on the 30 point Folstein
mini-mental state score annually), approximately 30% of AD
cases have a prolonged stable initial plateau phase lasting
several years, as described in Haxby et al. (1992), herein
incorporated by reference. A subgroup of patients has a
fulminant, rapidly progressive downhill course over several
years, as described in Mann et al. (1992), herein incorporated
by reference. Other patients (about 10% of cohorts) remain
slowly progressive, showing only gradual decline from year to
year, as described in Grossi et al. (1988), herein incorporated
by reference. The pathological, chemical and molecular bases
of this heterogeneity remain undetermined. Recognition of the

variability of AD progression represents an important clinical
insight, and may explain the diagnostic difficulties presented
by "atypical" cases.

   Attempts at predicting the onset of AD or monitoring
5 its progression have met with limited success. While in
certain cases, there is a familial manifestation of the
disease, it appears that the majority of AD cases are
non-familial, and until recently (see below), no simple genetic
marker for the disease had been determined. Much research has
10 focused on the protein beta-amyloid, deposits of which are
found in the brains of AD victims.

   However, recently, as described in our related US
Patent (No. 6,210,895; April 3, 2001) and publication (Schipper
et al., 2000), both herein incorporated by reference, we have
15 devised a diagnostic method, useful in the prediction,
diagnosis, and prognostication of AD, AACD/MCI, and related
neurological diseases. This diagnostic method is based on the
determination that patients suffering from AD have a
significantly lower concentration of heme oxygenase-1 (HO-1) in
20 their lymphocytes and plasma, and, accordingly, a significantly
lower concentration of ribonucleotide sequences encoding HO-1
in their lymphocytes.

   HO-1: Heme oxygenase-1 (HO-1) is an enzyme that
catalyses the rapid degradation of heme to biliverdin in brain
25 and other tissues. This 32 kDa member of the heat shock
protein superfamily contains a heat-shock element in its
promoter region and is rapidly up regulated in response to
oxidative stress, metal ions, amino acid analogues, sulfhydryl
agents, and hyperthermia. In response to oxidative stress,
30 induction of HO-1 may result in protection of cells by
catabolizing pro-oxidant metalloporphyrins, such as heme, into
bile pigments (biliverdin, bilirubin), with free radical
scavenging capabilities. Heme and other intracellular ferrous
iron chelates are capable of converting hydrogen peroxide to
35 the highly cytotoxic hydroxyl radical.

Using immunostaining techniques in conjunction with
laser scanning confocal microscopy, intense HO-1
immunoreactivity in neurons and astrocytes of post-mortem
hippocampus and temporal cortex derived from AD subjects has
5   been observed, whereas neural HO-1 staining was faint or non-
existent in the hippocampus and temporal cortex of control
specimens matched for age and post-mortem interval, as noted in
Schipper et al. (1995), herein incorporated by reference.
Furthermore, consistent co-localization of HO-1 to
10   neurofibrillary tangles and senile plaques in the AD specimens
has been demonstrated. Finally, robust 32 kDa bands
corresponding to HO-1 were observed by Western blotting of
protein extracts derived from AD temporal cortex and
hippocampus after SDS-PAGE, whereas control HO-1 bands were
15   faint or absent. These results indicate that HO-1 is
significantly over-expressed in neurons and astrocytes of AD
hippocampus and cerebral cortex relative to control brains and
support the contention that AD-affected tissues are
experiencing chronic oxidative stress.

20   **AACD/MCI:** AACD and MCI are terms used to identify
individuals who experience a cognitive decline that falls short
of dementia. These terms are equivalent, MCI being a more
recently adopted term, and are used interchangeably throughout
this application. Satisfaction of criteria (World Health
25   Organization) for this diagnosis requires a report by the
individual or family of a decline in cognitive function, which
is gradual, and present at least 6 months. There may be
difficulties across any cognitive domains (although memory is
impaired in the vast majority of cases), and these must be
30   supported by abnormal performance on quantitative cognitive
assessments for which age and education norms are available for
relatively healthy individuals (ie., the patient is compared to
normal subjects his/her own age). Performance must be at least
1 SD below the mean value for the appropriate population on
35   such tests. Neither dementia, nor significant depression or
drug effects may be present. No cerebral or systemic disease
or condition known to cause cerebral cognitive dysfunction may
be present. In Applicant's experience, all patients who were

classified as CDR.5 ("questionable dementia") on the Clinical
Dementia rating scale and who met these exclusions, also met
the criteria for AACD/MCI. About 1/3 of Alzheimer's patients
have had a clearly definable period of isolated memory deficit
5   which preceded their more global cognitive decline, as noted by
Haxby et al. (1992), herein incorporated by reference. Using
AACD/MCI criteria which look at other domains in addition to
memory, the percentage with an identifiable prodrome is likely
higher. Fortunately, not all AACD/MCI individuals seem to
10 decline. It appears that a significant number of these
subjects show a stable, non-progressive memory deficit on
testing.

        Related Disorders: Determining HO-1 concentration can
also assist in predicting, diagnosing, or prognosticating other
15 dementing diseases having similar manifestations and/or in
distinguishing such diseases from AD. Such other diseases
include Parkinson disease with dementia, Progressive
Supranuclear Palsy, Vascular (i.e. multi-infarct) Dementia,
Lewy Body Dementia, Huntington's Disease, Down's syndrome,
20 normal pressure hydrocephalus, corticobasal ganglionic
degeneration, multisystem atrophy, head trauma, neurosyphilis,
Creutzfeld-Jacob disease and other prion diseases, HIV and
other encephalitides, and metabolic disorders such as
hypothyroidism and vitamin B12 deficiency. The method may also
25 prove useful in differentiating the "pseudodementia" of
depression from Alzheimer disease.

        The determination of a relationship between HO-1
levels and AD represents a very significant advance in this
field, and may be utilized for the development of methods of
30 predicting, diagnosing in its very early stage, and
prognosticating AD and other dementing diseases. However,
identification of the factor(s) and mechanism(s) which control
HO-1 expression in the normal versus the diseased state are
needed, to provide even earlier diagnosis, as well as
35 therapeutic methods and reagents or substances, and methods and
reagents for the study of AD and other dementing diseases. In
addition, the reduction or absence of HO-1 in patients
suffering from AD represents a negative test, and, particularly

for the purposes of diagnosis, it would be more desirable to
have a positive indicator of disease, i.e. a factor whose
presence (rather than absence) correlates with disease.
Further, the decrease in HO-1 expression may represent an
5   effect, rather than a cause of AD and other dementing diseases,
therefore the identification of factor(s) and mechanism(s)
which control HO-1 expression in the normal versus the diseased
state are also needed to identify components and events which
have an active causative role in the onset and progression of
10   these diseases.

SUMMARY OF THE INVENTION

It is a goal of the present invention to provide
improved methods for predicting, diagnosing, prognosticating
and/or treating AD and other dementing diseases, as well as
15   methods and reagents to facilitate the study of the cause and
progression of these diseases.

Advantageously, embodiments of this invention provide
an easily administered blood or cerebrospinal fluid test which
is used to predict, diagnose, or prognosticate AD and other
20   dementing diseases.

One aspect of the present invention is a heme
oxygenase-1 suppressor (HOS) factor, wherein said factor
attenuates the increase in the level of heme oxygenase-1
(HO-1). In an embodiment, such an increase occurs in response
25   to exposure to an experimental agent or treatment which is
capable of increasing the level of HO-1. For example, such
experimental agents or treatments comprise exposure to any one
or more of oxidative stress, metal ions, amino acid analogues,
sulfhydryl agents (e.g., cysteamine, homocysteine),
30   interleukin-1β, tumour necrosis factor-α (TNF-α) and
hyperthermia.

Another aspect of the present invention is a method
for assessing dementing diseases in a patient which comprises:
determining the level of heme oxygenase-1 suppressor (HOS)
35   factor or activity, in tissue or a body fluid obtained from a
patient; and comparing said level of HOS factor or activity

with the corresponding level of HOS factor or activity in corresponding tissue or body fluid obtained from at least one control person, whereby if said level of HOS factor or activity is greater than said corresponding level of HOS factor or

5　activity in said tissue or body fluid obtained from at least one control person then said patient suffers from a dementing disease; wherein such method is used to predict the onset of, diagnose, or prognosticate dementing diseases.

　　　　Yet another aspect of the present invention is a

10　diagnostic method for differentiating, in a patient suffering from a dementing disease, between a dementing disease which is HO-1-dependent and a dementing disease which is HO-1-independent, said method comprising: determining the level of heme oxygenase-1 suppressor (HOS) factor or activity, in tissue

15　or a body fluid obtained from a patient suffering from a dementing disease; and comparing said level of HOS factor or activity with the corresponding level of HOS factor or activity in corresponding tissue or body fluid obtained from at least one control person, whereby if said level of HOS factor or

20　activity differs significantly from said corresponding level of HOS factor or activity in said tissue or body fluid obtained from at least one control person then said patient suffers from a dementing disease which is HO-1-dependent, and if said level of HOS factor or activity does not differ significantly from

25　said corresponding level of HOS factor or activity in said tissue or body fluid obtained from at least one control person then said patient suffers from a dementing disease which is HO-1-independent.

　　　　In an embodiment, another aspect of the present

30　invention is a method for differentiating the pseudodementia of depression from other dementing diseases in a patient which comprises: determining the level of heme oxygenase-1 suppressor (HOS) factor or activity, in tissue or body fluid obtained from a patient; and comparing said level of HOS factor

35　or activity with the corresponding level of HOS factor or activity in corresponding tissue or body fluid obtained from at least one control person; whereby if said level of HOS factor or activity is greater than said corresponding level of HOS

factor or activity in said corresponding tissue or body fluid
obtained from at least one control person then said patient
suffers from a dementing disease other than the pseudodementia
of depression; wherein such method is used to differentiate
5   the pseudodementia of depression from other dementing diseases.

The dementing diseases assessed using the methods
described above include, but are not limited to, Alzheimer
Disease, Age-Associated Cognitive Decline, Mild Cognitive
Impairment, Parkinson disease with dementia, Progressive
10 Supranuclear Palsy, Vascular (i.e. multi-infarct) Dementia,
Lewy Body Dementia, Huntington's Disease, Down's syndrome,
normal pressure hydrocephalus, corticobasal ganglionic
degeneration, multisystem atrophy, head trauma, neurosyphilis,
Creutzfeld-Jacob disease and other prion diseases, HIV and
15 other encephalitides, and metabolic disorders such as
hypothyroidism and vitamin B12 deficiency. Further, as noted
above, the methods may also prove useful in differentiating the
"pseudodementia" of depression from Alzheimer disease.

Examples of the above mentioned tissue or body fluids
20 include, but are not limited to, blood, plasma, lymphocytes,
cerebrospinal fluid, urine, saliva, epithelia, and fibroblasts.

The above-mentioned control tissue or body fluid, for
example, may be obtained from at least one normal age-matched
control person or from the patient at another time, in an
25 embodiment, at an earlier time.

Yet another aspect of the present invention is a
method for assaying the level of heme oxygenase-1 (HO-1)
suppressor (HOS) factor or activity in a sample which
comprises: exposing the sample to a cell culture; subjecting
30 the cell culture to exposure to an experimental agent or
treatment which may increase the level of HO-1 protein or mRNA
encoding HO-1; determining the level of HO-1 protein or mRNA
encoding HO-1; and comparing said level of HO-1 protein or mRNA
encoding HO-1 with a corresponding control level of HO-1
35 protein or mRNA encoding HO-1;

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whereby the level of said HO-1 protein or mRNA encoding HO-1 inversely correlates with the level of HOS factor or activity.

5   The present invention also provides evidence for the existence of a putative heme oxygenase-1 (HO-1) suppressor (HOS) factor in the samples derived from a patient suffering a dementing disease, as well as a partially purified fraction comprising HOS activity and a corresponding putative HOS factor.

10   Accordingly, a further aspect of the present invention is a method for assaying the level of heme oxygenase-1 (HO-1) suppressor (HOS) factor or activity in a sample which comprises: exposing the sample to a cell culture; subjecting the cell culture to exposure to an experimental

15 agent or treatment which may increase the level of HO-1 protein or mRNA encoding HO-1; determining the level of HO-1 protein or mRNA encoding HO-1; and comparing said level of HO-1 protein or mRNA encoding HO-1 with a corresponding control level of HO-1 protein or mRNA encoding HO-1; whereby the level of said HO-

20 1 protein or mRNA encoding HO-1 inversely correlates with the level of HOS factor or activity.

The above-mentioned corresponding control level of HO-1 protein or mRNA may be obtained, for example, by assaying the level of HO-1 protein or mRNA in a corresponding cell

25 culture which has been subjected to exposure to the above-mentioned experimental agent or treatment, but has not been exposed to the above-mentioned sample prior to exposure to the above-mentioned experimental agent or treatment.

Additional aspects of the present invention are

30 polyclonal and monoclonal antibodies which recognize the HOS factor, as well as hybridoma cells which produce the latter monoclonal antibodies.

Yet a further aspect of the present invention is a method for assaying the level of heme oxygenase-1 (HO-1)

35 suppressor (HOS) factor or activity in a sample comprising: exposing said sample to an antibody which recognizes the HOS

factor; isolating immune complexes; and determining the level of HOS factor or activity in the immune complex.

Since HOS affects the levels of HO-1 mRNA and protein, therefore the invention also contemplates a method for assaying the level of HOS activity or factor using a reporter construct comprising transcriptional regulatory element(s) (e.g., a promoter region) of the HO-1 gene operably linked to a suitable reporter gene.

Accordingly, a further aspect of the present invention is a method for assaying the level of heme oxygenase-1 (HO-1) suppressor (HOS) activity in a sample comprising: exposing said sample to a reporter construct, wherein said reporter construct comprises the HO-1 promoter region and a reporter gene, wherein said reporter gene encodes a protein which possesses a detectable reporter activity; determining the level of said reporter activity, and comparing said level of said reporter activity with a corresponding control level of said reporter activity; whereby the level of said reporter activity inversely correlates with the level of HOS factor or activity.

The above-mentioned control level of reporter activity may be obtained, for example, by measuring the reporter activity produced by a corresponding reporter construct that has not been exposed to the above-mentioned sample.

The HOS activity of the present invention may also be used for the elucidation of other factors and mechanisms involved in the onset and progression of AD and other dementing diseases. These factors and mechanisms may yield therapeutic agents and methods, as well as contribute to our understanding of the molecular events which are involved in the onset and progression of AD and other dementing diseases.

Therefore, a further aspect of the present invention is a method for screening a candidate compound for the presence of an inhibitor or activator of HOS activity or HOS factor comprising: exposing said candidate compound to a sample known

to comprise HOS activity or HOS factor; assaying the level of
HOS activity or HOS factor using a method selected from the
group consisting of: (a) a method for assaying the level of
heme oxygenase-1 (HO-1) suppressor (HOS) factor or activity in

5   a sample which comprises: exposing the sample to a cell
culture; subjecting the cell culture to exposure to an
experimental agent or treatment which may increase the level of
mRNA encoding HO-1; determining the level of HO-1 protein or
mRNA encoding HO-1; and comparing said level of HO-1 protein or

10 mRNA encoding HO-1 with a corresponding control level of HO-1
protein or mRNA encoding HO-1; whereby the level of said HO-1
protein or mRNA encoding HO-1 inversely correlates with the
level of HOS factor or activity; (b) a method for assaying the
level of heme oxygenase-1 (HO-1) suppressor (HOS) factor or

15 activity in a sample comprising: exposing said sample to an
antibody which recognizes the HOS factor; isolating immune
complexes; and determining the level of HOS factor or activity
in the immune complex; and (c) a method for assaying the level
of heme oxygenase-1 (HO-1) suppressor (HOS) factor or activity

20 in a sample comprising: exposing said sample to a reporter
construct, wherein said reporter construct comprises the HO-1
promoter region and a reporter gene, wherein said reporter gene
encodes a protein which possesses a detectable reporter
activity; and determining the level of said reporter activity;

25 and comparing said level of said reporter activity with a
corresponding control level. of said reporter activity; whereby
the level of said reporter activity inversely correlates with
the level of HOS factor or activity; and comparing said level
of HOS activity or HOS factor with a corresponding control

30 level of HOS activity or HOS factor in a corresponding control
sample, wherein said control sample comprises said sample
known to comprise HOS activity that has not been exposed to
said candidate compound.

A further aspect of the present invention is a
35 commercial package comprising means for determining the level
of heme oxygenase-1 (HO-1) suppressor (HOS) factor or activity,
in tissue or body fluid obtained from a patient, and
instructions for comparing said level of HOS factor or activity

with an established standard of the corresponding HOS activity
in corresponding control tissue or body fluid. Such control
tissue or body fluid, for example, may be obtained from at
least one normal age-matched control person or from the patient
5   at another time, in an embodiment, at an earlier time.

Since levels of HO-1 mRNA, protein and/or activity as
well as HOS factor and/or activity may be altered in patients
suffering from a dementing disease, inhibitors or activators of
HOS factor or HOS activity represent potential substances or
10 compounds which may be utilized for the treatment of a
dementing disease.

Accordingly, a further aspect of the present
invention is a compound for the treatment of a dementing
disease, wherein the compound alleviates the dementing disease
15 by increasing or decreasing the level of heme oxygenase-1
(HO-1) mRNA, protein or activity.

A further aspect of the present invention is a
compound for the treatment of a dementing disease, wherein the
compound alleviates the dementing disease by increasing or
20 decreasing the level of heme oxygenase-1 (HO-1) suppressor
(HOS) factor or activity.

Yet a further aspect of the present invention is a
pharmaceutical composition for the treatment of a dementing
disease which comprises the substance or compound described
25 above in admixture with a suitable pharmaceutically acceptable
diluent or carrier.

Yet a further aspect of the present invention is a
method of treating a dementing disease in a patient, comprising
administering to said patient the compound or pharmaceutical
30 composition described above in an amount effective to treat a
dementing disease, wherein said method results in the
alleviation of the dementing disease by increasing or
decreasing the level of heme oxygenase-1 (HO-1) mRNA, protein
or activity.

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Yet a further aspect of the present invention is a
method of treating a dementing disease in a patient, comprising
administering to said patient the compound or pharmaceutical
composition described above in an amount effective to treat a
5   dementing disease, wherein said method results in the
alleviation of the dementing disease by increasing or
decreasing the level of heme oxygenase-1 (HO-1) suppressor
(HOS) factor or activity.

Yet a further aspect of the present invention is a
10   use of the above-mentioned compound or pharmaceutical
composition for the treatment of a dementing disease.

Yet a further aspect of the present invention is a
commercial package containing as an active pharmaceutical
ingredient the compound or pharmaceutical composition described
15   above together with instructions for its use in the treatment
of a dementing disease.

The substance or compound, composition, method and
commercial package noted above may, for example, be utilized
for the treatment of a dementing disease selected from the
20   group consisting of Alzheimer Disease, Age-Associated Cognitive
Decline, Mild Cognitive Impairment, Parkinson disease with
dementia, Progressive Supranuclear Palsy, Vascular (i.e. multi-
infarct) Dementia, Lewy Body Dementia, Huntington's Disease,
Down's syndrome, normal pressure hydrocephalus, corticobasal
25   ganglionic degeneration, multisystem atrophy, head trauma,
neurosyphilis, Creutzfeld-Jacob disease and other prion
diseases, HIV and other encephalitides, and metabolic disorders
such as hypothyroidism and vitamin B12 deficiency.

BRIEF DESCRIPTION OF THE DRAWINGS

30   Figure 1 is a Northern analysis of HO-1 mRNA
implicating the presence of a circulating HO-1 suppressor (HOS)
factor in sporadic AD, as described in Example 1. Control
GAPDH bands used to ensure uniformity of RNA loading are
depicted below the HO-1 bands.

Figure 2 depicts tabular results of studies of demographics and HOS activity in normal young control (NYC), normal elderly control (NEC), mild cognitive impairment (MCI) and sporadic Alzheimer disease (AD) subjects, as described in

5   Example 2. Suppression by 24h incubation in human plasma of CSH-induced ($880\mu M \times 6$ h) glial HO-1 mRNA band (Northern blot) relative to CSH-treated astrocytes grown in standard culture media; 0=0-25% suppression, 1=26-50% suppression, 2=51-75% suppression, 3=76-100% suppression. HOS = HOS activity; MMSE

10   = Folstein Mini-mental State Exam Score; Cortisol = Plasma cortisol levels (nMol/L); AD Meds = cholinesterase inhibitors used for treatment of Alzheimer disease; E400 and E800 = 400 and 800 units vitamin E, respectively; C500 = 500 mg vitamin C.

Figure 3 depicts graphical results of HOS activity in

15   normal control (NC), mild cognitive impairment (MCI) and sporadic Alzheimer disease (AD) subjects. HOS activity=percentage suppression (quartiles) by 24h incubation in human plasma of CSH-induced ($880\mu M \times 6$ h) glial HO-1 mRNA band (Northern blot) relative to CSH-treated astrocytes grown

20   in standard culture media, as described in Example 3.

Figure 4 depicts analysis of plasma cortisol levels (mean $\pm$ SD) in normal control (NC), mild cognitive impairment (MCI) and sporadic Alzheimer disease (AD) subjects (panel A), as described in Example 4. ( )=number of cases per group.

25   Differences between groups are not statistically significant (1-way ANOVA). Correlation between plasma cortisol levels and HOS activity in the MCI (panel B) and AD (panel C) groups is not significant (linear regression analysis).

Figure 5 is a Northern analysis of HO-1 mRNA

30   demonstrating the effects of sample storage time and antioxidant exposure on plasma HOS activity, as described in Example 8. C=Control (untreated) astrocyte cultures, CSH=cysteamine-treated astrocyte culture, AD=Alzheimer, MCI=Mild Cognitive Impairment, NEC=normal elderly control,

35   N=normal subject on antioxidants. Control GAPDH bands are used as noted in Figure 1.

Figure 6 is a Northern analysis of HO-1 mRNA demonstrating the effects of plasma dilution on HOS activity, as described in Example 6.

Figure 7 is a Northern analysis of HO-1 mRNA
5   demonstrating the effect of heat treatment on HOS activity, as described in Example 7. Control GAPDH bands are used as noted in Figure 1.

Figure 8 is a Northern analysis of HO-1 mRNA demonstrating the partial purification of HOS factor by
10 heparin-agarose chromatography, as described in Example 8. Control GAPDH bands are used as noted in Figure 1.

Figure 9 is a Northern analysis of HO-1 mRNA demonstrating further HOS purification of the heparin agarose eluate by Concanavalin-A (Con-A) Agarose affinity column
15 chromatography, as described in Example 9. Control GAPDH bands are used as noted in Figure 1.

Figure 10 is a Northern analysis of HO-1 mRNA demonstrating further HOS purification of the heparin agarose-conconavalin A eluate derived from 4 pooled AD plasma samples
20 (29 cc starting material) on a Superose™ 12 HR FPLC Column, as described in Example 10. Control GAPDH bands are used as noted in Figure 1.

Figure 11 presents graphical results of relative protein concentrations in Superose™ 12 HR FPLC Column fractions
25 derived from pooled AD plasma samples described in Fig. 10, as described in Example 10. Arrow denotes protein concentration in fraction (number 20-22) exhibiting robust HOS activity.

Figure 12 depicts results of a chromatogram from a function test of Superose™ 12 HR FPLC 1-cm diameter column
30 (Catl. # 17-0538-01, Lot # 8283034) [Amersham Pharmacia Biotech, Inc Quebec Canada] using standard protein mixtures, as described in Example 10.

Figure 13 is a Northern analysis of HO-1 mRNA demonstrating the effects of NEC and AD plasma on astrocyte HO-

1 mRNA induction by multiple stimuli. The HOS bioassay was
performed as described for Figure 1. Northern blots for HO-1
mRNA (top) and respective GAPDH mRNA (bottom) are shown.
Control GAPDH bands are used as noted in Figure 1.

5   **DESCRIPTION OF THE EMBODIMENTS OF THE INVENTION**

            Applicant has devised an improved diagnostic method,
useful in the prediction, diagnosis, and prognostication of AD,
AACD/MCI, and related neurological diseases, as well as methods
and reagents which are useful in the treatment and study of AD,
10 AACD/MCI, and related neurological diseases. These methods are
based on the discovery that patients suffering from AD have an
activity and corresponding factor in their plasma which
significantly suppresses the expression of heme oxygenase-1
(HO-1). This HO-1 suppressor activity is assayed via the
15 inability to upregulate the concentration of nucleotide
sequences encoding HO-1, in response to exposure to a suitable
experimental agent or treatment, in a suitable cell culture
system pre-incubated with a tissue or body fluid derived from
patients suffering from AD or other dementing diseases. This
20 suppressor activity and corresponding factor shall be referred
to as HO-1 suppressor (HOS) activity and factor, respectively.

            Applicant has identified an activity, namely HOS
activity, which is present in tissue or body fluids derived
from patients suffering from AD as well as possibly those
25 suffering from other dementing diseases, but is absent in
normal age-matched control subjects. This activity may be
detected in a tissue or body fluid obtained from these
patients. Examples of possible sources of suitable tissue or
body fluids include blood, plasma, lymphocytes, cerebrospinal
30 fluid, urine, saliva, epithelia (such as skin epithelia) and
fibroblast cell lines derived from patients.

            An aspect of the invention is a HOS activity, which
is an activity that suppresses the upregulation of HO-1
expression. Such upregulation occurs, for example, following
35 exposure to an experimental agent or treatment which is, in the
absence of HOS activity, capable of increasing HO-1 expression,

as detected.by increases in HO-1 protein or HO-1 mRNA. In
patients suffering from AD, as well as possibly those suffering
from other dementing diseases, HOS activity suppresses the
expression of HO-1, which is expressed at significantly higher
5   levels in lymphocytes and possibly other non-neural tissue or
body fluids in normal aged-matched control subjects.

        A further aspect of the invention is a method of
assaying HOS activity in a sample. Examples of possible
sources of suitable samples include tissues and body fluids
10 such as, for example, blood, plasma, lymphocytes, cerebrospinal
fluid, urine, saliva, epithelia and fibroblast cell lines
derived from patients, or fractions derived from these samples.
The assay involves exposing the sample to be tested to a cell
culture that is capable of undergoing an induction in HO-1
15 expression in response to exposure to a certain experimental
agent or treatment. An example of such a cell culture is a rat
astroglial culture, however, many other useful possibilities
exist. Examples of such exposure to an experimental agent or
treatment include exposure to oxidative stress, metal ions,
20 amino acid analogues, sulfhydryl agents, interleukin-1β, tumour
necrosis factor-α (TNF-α) and hyperthermia. Examples of
suitable sulfhydryl reagents include, but are not limited to,
cysteamine and homocysteine. Following such exposure to an
experimental agent or treatment, the level of HO-1 protein or
25 HO-1 mRNA may be detected using suitable methods. The level of
HO-1 may for example be detected by an immunoassay. The level
of HO-1 mRNA may for example be detected by Northern analysis
using an appropriate probe(s). Detection of HO-1 mRNA of
greater sensitivity may be achieved for example using the
30 reverse transcriptase-polymerase chain reaction (RT-PCR)
method, described in Abraham (1998) and Mawal et al. (2000),
both herein incorporated by reference. The activity assay may
be adapted to a large scale level for analyzing a large number
of samples simultaneously, possibly in a suitable array format,
35 possibly with the automated execution (e.g., by robotics) of
some or all of the steps therein.

A further possibility may be the development of a reporter-based assay for assaying HOS activity. Such an assay may involve the preparation of a suitable reporter construct, e.g. comprising a transcriptional regulatory element, such as

5 the 5' untranslated promoter region, of the HO-1 gene, operably linked to a suitable reporter gene, i.e., capable of regulating the expression of a suitable reporter gene. Such a construct may additionally comprise the 3' untranslated region of the HO-1 gene or another suitable 3' sequence. In another

10 embodiment, the construct may comprise an in frame fusion of a suitable reporter gene within the open reading frame of the HO-1 gene. The reporter gene may be chosen as such to facilitate the detection of its expression, e.g. by the detection of the presence and/or activity of its gene product.

15 Many such suitable reporters may be used, which provide detectable signals. Most preferred embodiments in this class are those that provide a conveniently detectable signal, which may be detected by, for example, spectroscopic methods. Examples of suitable reporter genes include those encoding

20 luciferase, beta-galactosidase, green fluorescent protein, alkaline phosphatase, chloramphenicol acetyltransferase, as well as others. Such a reporter construct may be introduced into a suitable system capable of exhibiting an increase in the level of expression of the reporter gene in response to

25 exposure to an experimental agent or treatment which is capable of increasing HO-1 expression as noted above. Such an assay would also be adaptable to a possible large scale, high-throughput, automated format as noted above, and would allow more convenient detection due to the presence of its reporter

30 component.

Using methods of assaying HOS activity as described above, applicant has determined that the level of HOS activity in a sample decreases with the increasing dilution of the sample, suggesting that HOS activity is attributed to the

35 presence of a corresponding HOS factor. Using the same assay methods, applicant has further determined that pre-heating the sample to be tested abrogates HOS activity, suggesting that HOS activity is attributed to a protein or complex of proteins.

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Since, to applicant's knowledge, glucocorticoids are the only
known suppressors of HO-1 expression (Lavrovsky et al., 1996;
Deramaudt et al., 1999), the discovery of a protein-like HOS
factor is novel. Applicant has further demonstrated that

5   cortisol levels are not increased in AD or MCI samples with
respect to normal samples, thus demonstrating that suppression
of HO-1 expression in AD and MCI samples is not attributed to
glucocorticoids, but rather, is a result of the activity of a
(non-glucocorticoid) HOS factor.

10   Applicant has accomplished a partial purification of HOS
activity and therefore HOS factor using one or multiple
chromatographic methods in sequential fashion. An example of a
suitable chromatographic method is affinity chromatography
using a heparin-agarose matrix or a concanavallin-A (Con-A)

15   agarose matrix or gel filtration chromatography using for
example a Superose™-12 matrix. Applicant has accomplished
further purification of HOS factor using heparin-agarose,
concanavallin-A (Con-A) agarose and Superose™-12
chromatography, in sequence, further suggesting that HOS factor

20   comprises a protein or complex of proteins, and, based on
binding to the Con-A matrix, likely comprises a glycoprotein,
in an embodiment, a mannoprotein. This suggests that HOS
activity and the corresponding HOS factor may be obtained in a
more highly purified form using various chromatographic

25   methods. Such purification is for example shown in Figure 11,
where the peak of HOS activity elutes later that most of the
protein in the sample, thus indicating that the Superose™-12
column has removed the majority of protein contaminants from
the HOS factor-containing sample. Calibration of the column

30   using known protein molecular weight standards (Figure 12)
indicates that HOS factor is a protein or complex of proteins
having an approximate molecular weight in the range of 80-100
kDa, in an embodiment, having a molecular weight of
approximately 90 kDa. These data thus provide further support

35   that HOS factor is a protein-like molecule. Applicant has
further shown that HOS factor and associated HOS activity are
stable during prolonged storage.

Accordingly, the invention further provides a HOS
factor, as described above.

Applicant has further demonstrated that HOS activity
is not due to simple antioxidant behaviour, since both AD and
5   normal plasma exhibit equivalent levels of partial suppression
of the HO-1 mRNA response to a pro-oxidant, for example,
menadione. Further, typical doses of antioxidants have no
effect on the induction of HO-1 mRNA expression, and exposure
of multiple, high dose, antioxidants only results in partial
10   suppression.

A further aspect of the present invention is an
improved diagnostic method, potentially useful in the
prediction, diagnosis, and prognostication of AD, AACD/MCI, and
related neurological diseases. This diagnostic method is based
15   on the detection of HOS activity, using for example the assay
methods described above, in a tissue or body fluid obtained
from a patient. Because the presence of HOS activity precedes
any decrease in HO-1 expression in a patient, this diagnostic
method provides an even earlier diagnosis of AD, AACD/MCI, and
20   related neurological diseases. In addition, the
immunodetection of HOS factor or activity (see below) may
provide an improved method of diagnosis over the detection of
decreases in HO-1 expression using methods such as Northern
analysis or the reverse transcriptase-polymerase chain reaction
25   (RT-PCR) method, described in Abraham (1998) and Mawal et al.
(2000), both herein incorporated by reference. Further, the
correlation of the presence of HOS activity with the disease
state represents a positive test for diagnosis. This is more
desirable than a negative test, used for diagnosis based on the
30   reduction or absence of HO-1 expression in a patient suffering
from one of the dementing diseases described above.

It is known in the art that certain dementing
diseases, for example, AD, correlate with changes in HO-1
levels while others do not. Such dementing diseases may be
35   categorized as HO-1-dependent and HO-1-independent. As
described in the instant application, such changes in HO-1
levels are a result of changes in the levels of HOS factor or

activity. Therefore, the invention further relates to methods, reagents, compounds and commercial packages to differentiate a dementing disease which exhibits a significantly altered level of HO-1 protein, HO-1 mRNA, HOS factor, or HOS activity, i.e.,

5   an HO-1-dependent dementing disease, from a dementing disease which does not exhibit such a significantly altered level of HO-1 protein, HO-1 mRNA, HOS factor, or HOS activity, i.e., an HO-1-independent dementing disease. The term "significantly" as used here means that the levels are altered from control

10   levels beyond the range of experimental error, as known in the art.

        The HOS activity of the present invention may also be used to develop therapeutic agents and methods for the treatment of AD and other dementing diseases. Since the

15   appearance of HOS activity correlates with the presence of the disease state, the HOS activity and HOS factor is expected to play a causative role in the onset and/or progression of AD and other dementing diseases. Therefore, identification of factors or mechanisms which inhibit or activate HOS activity may be

20   utilized for the development of therapeutic agents and methods for the treatment of AD and other dementing diseases. If an increase in HOS activity is a causative event in the onset and/or progression of AD and other dementing diseases, an inhibitor of HOS activity is expected to have therapeutic

25   potential. Conversely, an activator of HOS activity is expected to represent an upstream causative agent of the onset and/or progression of AD and other dementing diseases, which may provide even earlier and improved methods of diagnosis. Further, all factors which effect HOS activity will lead to a

30   better understanding of the mechanisms of the onset and/or progression of AD and other dementing diseases, and ultimately contribute to the development of improved therapeutic methods and agents. In addition, other factors which affect levels of HO-1 mRNA, protein and activity are also useful to the

35   invention, similar to the above, and are thus a further aspect of the invention.

        Accordingly, it is a further aspect of the present invention to provide a HOS activity-based screening method to

identify putative compounds which either inhibit or augment HOS
activity. Such screening may be performed using for example
the HOS activity assays described above, and may be adapted to
a large scale, and possibly automated format. Such a method
5   may comprise exposing a known HOS activity-containing sample to
the compound to be tested, and subsequently determining the
level of HOS activity present, which is then compared to a
control sample that was not exposed to the compound to be
tested. In a high-throughput, automated format, this screening
10   method may be used for the rapid analysis of libraries
containing a large number of compounds for their effects on HOS
activity. In an embodiment, examples of such libraries include
chemical libraries prepared by combinatorial synthesis.

The partially purified fraction comprising HOS factor
15   and HOS activity, obtained, for example, from heparin-agarose
and/or Con-A agarose and/or Superose™-12 column chromatography,
may be used to immunize a small mammal, e.g., a mouse or a
rabbit, in order to raise antibodies which recognize this
activity. In an embodiment the above mentioned fraction is
20   obtained from sequential heparin-agarose, Con-A agarose and
Superose™-12 column chromatography. Accordingly, a further
aspect of the invention provides an antibody that recognizes
the HOS factor of the invention.

An antibody of the invention is either polyclonal or
25   monoclonal. Antibodies may be recombinant, e.g., chimeric
(e.g., constituted by a variable region of murine origin
associated with a human constant region), humanized (a human
immunoglobulin constant backbone together with hypervariable
region of animal, e.g., murine, origin), and/or single chain.
30   Both polyclonal and monoclonal antibodies may also be in the
form of immunoglobulin fragments, e.g., F(ab)'$_2$, Fab or Fab'
fragments. The antibodies of the invention are of any isotype,
e.g., IgG or IgA, and polyclonal antibodies are of a single
isotype or a mixture of isotypes.

35   Antibodies against the HOS factor of the present
invention are generated by immunization of a mammal with a
partially purified fraction comprising HOS factor. In an

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embodiment the above mentioned fraction is obtained from
sequential heparin-agarose, Con-A agarose and Superose™-12
column chromatography.   Such antibodies may be polyclonal or ·
monoclonal.   Methods to produce polyclonal or monoclonal

5   antibodies are well known in the art.   For a review, see Harlow
and Lane (1988) and Yelton et al. (1981), both of which are
herein incorporated by reference.   For monoclonal antibodies,
see Kohler and Milstein (1975), herein incorporated by
reference. ·

10   The antibodies of the invention, which are raised to
a partially purified fraction comprising HOS factor of the
invention, are produced and identified using standard
immunological assays, e.g., Western blot analysis, dot blot
assay, or ELISA (see, e.g., Coligan et al. (1994), herein

15   incorporated by reference).   The antibodies are used in
diagnostic methods to detect the presence of a HOS factor and
activity in a sample, such as a tissue or body fluid.   The
antibodies are also used in affinity chromatography for
obtaining a purified fraction comprising the HOS factor and

20   activity of the invention.

          Accordingly, a further aspect of the invention
provides (i) a reagent for detecting the presence of HOS factor
and activity in a tissue or body fluid; and (ii) a diagnostic .
method for detecting the presence of HOS factor and activity in

25   a tissue or body fluid, by contacting the tissue or body fluid
with an antibody of the invention, such that an immune complex
is formed, and by detecting such complex to indicate the
presence of HOS factor and activity in the sample or the
organism from which the sample is derived.

30   Those skilled in the art will readily understand that
the immune complex is formed between a component of the sample
and the antibody, and that any unbound material is removed
prior to detecting the complex.   It is understood that an
antibody of the invention is used for screening a sample, such

35   as, for example, blood, plasma, lymphocytes, cerebrospinal
fluid, urine, saliva, epithelia and fibroblasts, for the
presence of·HOS activity.

For diagnostic applications, the reagent (*i.e.*, the antibody of the invention) is either in a free state or immobilized on a solid support, such as a tube, a bead, or any other conventional support used in the field. Immobilization

5 is achieved using direct or indirect means. Direct means include passive adsorption (non-covalent binding) or covalent binding between the support and the reagent. By "indirect means" is meant that an anti-reagent compound that interacts with a reagent is first attached to the solid support.

10 Indirect means may also employ a ligand-receptor system, for example, where a molecule such as a vitamin is grafted onto the reagent and the corresponding receptor immobilized on the solid phase. This is illustrated by the biotin-streptavidin system. Alternatively, a peptide tail is added chemically or by

15 genetic engineering to the reagent and the grafted or fused product immobilized by passive adsorption or covalent linkage of the peptide tail.

Such diagnostic agents may be included in a kit which also comprises instructions for use. The reagent is labeled

20 with a detection means which allows for the detection of the reagent when it is bound to its target. The detection means may be a fluorescent agent such as fluorescein isocyanate or fluorescein isothiocyanate, or an enzyme such as horse radish peroxidase or luciferase or alkaline phosphatase, or a

25 radioactive element such as ^{125}I or ^{51}Cr.

Accordingly, a further aspect of the invention provides a process for purifying, from a tissue or body fluid, the HOS factor of the invention, which involves carrying out antibody-based affinity chromatography with the tissue or body

30 fluid, wherein the antibody is an antibody of the invention.

For use in a purification process of the invention, the antibody is either polyclonal or monoclonal, and preferably is of the IgG type. Purified IgGs are prepared from an antiserum using standard methods (see, *e.g.*, Coligan *et al.*

35 (1994), herein incorporated by reference). Conventional chromatography supports, as well as standard methods for

grafting antibodies, are described in, *e.g.*, Harlow and Lane (1988), herein incorporated by reference, and outlined below.

Briefly, a tissue or body fluid, such as plasma from a patient suffering from AD, preferably in a buffer solution,

5   is applied to a chromatography material, preferably equilibrated with the buffer used to dilute the tissue or body fluid so that the HOS factor of the invention (*i.e.*, the antigen) is allowed to adsorb onto the material. The chromatography material, such as a gel or a resin coupled to an

10   antibody of the invention, is in either a batch form or a column. The unbound components are washed off and the antigen is then eluted with an appropriate elution buffer, such as a glycine buffer or a buffer containing a chaotropic agent, *e.g.*, guanidine HCl, or high salt concentration (*e.g.*, 3 M MgCl$_2$).

15   Eluted fractions are recovered and the presence of the antigen is detected, *e.g.*, by measuring the absorbance at 280 nm.

A further aspect of the present invention is a diagnostic imaging method, which comprises introducing into a biological system, an antibody of the invention, which is used

20   in conjunction with an appropriate detection system to identify areas where HOS factor or activity is present or absent.

The following examples are provided in order to illustrate the methods of the present invention and are not meant to limit the scope of the invention.

25   **Example 1: Determination of the presence of HOS activity in plasma derived from AD patients**

Whole blood is collected from normal elderly (N1, N2) subjects or patients with probable sporadic AD (AD1, AD2) in heparinized tubes. This is then layered over a Ficoll Paque™

30   density gradient and centrifuged at 1800 rpm for 20 minutes. The top plasma layer is then collected and saved for incubation with rat astroglia as described below. The lymphocyte fractions are collected and used for the isolation of mRNA for Northern analysis as described below.

Determination of lymphocyte HO-1 mRNA levels:

Lymphocyte fractions were obtained by differential
centrifugation of whole blood on Ficoll Paque™ gradients as
described above. Cytoplasmic RNA was isolated from the
5   lymphocytes using an acid guanidinium thiocyanate-phenol-
chloroform extraction method, as described by Chomezynski
et al. (1997), herein incorporated by reference. Six
micrograms of RNA was denatured and size-separated by
electrophoresis on 1% agarose/formaldehyde gels. RNA integrity
10 was confirmed by ethidium bromide staining. The RNA was
transferred onto Hybond-N™ filter paper and covalently cross-
linked to the membrane by UV light for two minutes. The
hybridization probe (HO-1; 1.0kb) was prepared by random
priming using the Random Primer DNA Labelling System, as
15 described by Feinberg et al. (1984), herein incorporated by
reference. Prehybridization was performed for 12 hours at 42°C
in a buffer containing formamide deionized, 5 x Denhardt's
reagent, 6 x SSPE and 0.5% SDS. The hybridization buffer
consisted of the prehybridization buffer without 5 x Denhardt's
20 reagent, and ^{32}P-labelled denatured DNA probe, as described in
Noonberg et al. (1994), herein incorporated by reference.
Equal loading of RNA was confirmed by hybridization with a cDNA
for the (housekeeping) gene, glyceraldehyde-3-phosphate
dehydrogenase (GAPDH). All washes were performed under
25 stringent conditions (1 x SSC and 0.2% SDS for 45 minutes at
room temperature, 0.4 x SSC and 0.2% SDS for 15 minutes at
65°C). The RNA hybridizing with cDNA probes was visualized by
autoradiography using an intensifying screen at -80°C, as
described in Church et al. (1984), herein incorporated by
30 reference.

As noted in our related US Patent (No. 6,210,895;
April 13, 2001) and publication (Schipper et al., 2000), both
herein incorporated by reference, and as reiterated in Panel A
of Figure 1, lymphocytes isolated from normal subjects N1 and
35 N2 exhibit significant levels of HO-1 mRNA (lanes 1 and 2),
which is not detectable in lymphocytes isolated from AD
patients AD1 and AD2 (lanes 3 and 4).

Assay of plasma HOS activity via the induction of HO-1 expression upon cysteamine (CSH) treatment of rat astroglia:

Brain cell cultures: Rat astroglia were prepared as
5 described in Schipper et al. (1999), herein incorporated by reference, as follows:

Pregnant Sprague-Dawley rats were obtained from Charles River Breeding Farms. Primary neural cell cultures were prepared from 1-day old neonates by mechanoenzymatic
10 dissociation of cerebral tissue or body fluid as previously described by Chopra et al., (1997), herein incorporated by reference. Cells were grown in Ham's F-12 and high-glucose DMEM (50:50 vol/vol) supplemented with 10 mM HEPES. 5% heat-inactivated horse serum, 5% heat-inactivated fetal bovine
15 serum, and penicillin/streptomycin (50 U/ml and 50 μg/ml, respectively). The cells were plated in 75-cm^2 tissue or body fluid culture flasks at a density of 1 x 10^6 cells/ml. Cultures were incubated at 37°C in humidified 95% air/5% CO$_2$ for 6h, at which time they were vigorously shaken 20-30 times with
20 replacement of fresh medium to remove adherent oligodendroglia and microglia from the astrocytic monolayers. The cultures were then incubated under the above-mentioned conditions for 6 days, at which time >98% of the cells composing the monolayer were astroglia, as determined by immunohistochemical labeling
25 for the astrocyte-specific marker glial fibrillary acidic protein, as described by Chopra et al. (1995), herein incorporated by reference. These astroglia cultures were grown under different conditions and subjected to different treatments (see below), and subsequently mRNA was isolated for
30 Northern analysis of HO-1 mRNA levels as described in Schipper et al. (1999), herein incorporated by reference, as follows:

RNA isolation and Northern analysis: Cultured astrocytes were harvested using a rubber policeman, and cytoplasmic RNA was isolated using an acid guanidinium
35 thiocyanate/phenol/chloroform extraction method, as described by Chomczynski and Sacchi (1987), herein incorporated by reference. Ten micrograms of RNA was denatured and size-

separated by electrophoresis on 1% agarose/formaldehyde gels.
RNA integrity was confirmed by ethidium bromide staining. The
RNA was transferred onto Hybond-N™ filter paper and covalently
cross-linked to the membrane by UV light for 2 min. The
5 hybridization probe (HO-1; 1.0 kb) was prepared by random
primer-generated double-strand DNA probes using the random
primer DNA labeling system, as described by Feinberg and
Vogelstein (1984), herein incorporated by reference.
Prehybridization was performed for 12 h at 42°C in a buffer
10 containing formamide-deionized, 5X Denhardt's reagent,
6X saline-sodium phosphate-EDTA, and 0.5% sodium dodecyl
sulfate (SDS). The hybridization buffer consisted of the
prehybridization buffer without 5X Denhardt's reagent and
^{32}P-labeled denatured DNA probe, as described by Noonberg et al.
15 (1994), herein incorporated by reference. Equal loading of RNA
was confirmed by hybridization with a cDNA for the
(housekeeping) gene, glyceraldehyde-3-phosphate dehydrogenase
(GAPDH), or 18S mRNA. All washes were performed under
stringent conditions [1X saline-sodium citrate (SSC) and 0.2%
20 SDS for 45 min at room temperature, 0.4X SSC and 0.2% SDS for
15 min at 65°C, and 0.1X SSC and 0.2% SDS for 15 min at 65°C].
The RNA hybridizing with cDNA probes was SDS for 15 min at
65°C, and 0.1X SSC and 0.2% SDS for 15 min at 65°C]. The RNA
hybridizing with cDNA probes was visualized by autoradiography
25 using an intensifying screen at -80°C, as described by Church
and Gilbert (1984), herein incorporated by reference.
Resulting bands on the autoradiograph were analyzed using a
phosphorimager S1 densitometer. Densitometry data were
normalized by calculating the ratios of the HO-1 mRNA signals
30 to control GAPDH or 18S mRNA signals.

Figure 1, panel A: Northern blot of lymphocyte HO-1
mRNA (and control GAPDH mRNA) derived from 2 normal elderly
individuals (N1, N2) and 2 patients with probable sporadic AD
AD1, AD2). As noted in our related US Patent (No. 6,210,895;
35 April 13, 2001) and publication (Schipper et al., 2000), both
herein incorporated by reference, lymphocyte HO-1 mRNA bands
are visible in the controls (lanes 1 and 2), and not detectable

(lanes 3 and 4) in the AD subjects, indicating the presence of HOS activity in the latter.

Using the methods described above, Northern analysis of HO-1 mRNA levels of rat astroglia grown under different
5   conditions and subjected to different treatments was performed, the results of which are shown in panels B and C of Figure 1. Panel B: Control (unchallenged) rat astroglia grown in standard culture media for 6 days exhibit faint or no HO-1 mRNA bands (lanes 5-7). Cysteamine (CSH) treatment (880μM x 6hr)
10   induces robust HO-1 mRNA bands in these cells (lanes 8-10). Twenty-four hour incubation of the rat astroglia with the plasma derived from the same 2 normal subjects (N1, N2; lanes 11-12) and the 2 AD patients (AD1, AD2; lanes 13-14) noted above (see panel A) has no appreciable affect on baseline HO-1
15   mRNA levels. Panel C: In contrast to plasma derived from the 2 normal subjects (lanes 15-24), undiluted plasma from the 2 AD patients markedly suppresses the rat astroglial HO-1 mRNA response to CSH (lanes 25-27; 30-32). Dilution of the AD plasma (1:9 in standard culture media; "10%") greatly
20   diminishes its inhibitory effect on CSH-induced HO-1 mRNA expression (lanes 28-29; 33-34). Therefore, there exists in the plasma of AD patients an HOS activity, which is not present in the plasma of normal subjects, and which is assayable by the determination of HO-1 mRNA levels in rat astroglia incubated
25   with the relevant plasma sample and subjected to CSH treatment.

Example 2: Demographics and HOS activity in normal young control (NYC), normal elderly control (NEC), mild cognitive impairment (MCI) and sporadic Alzheimer disease (AD) subjects.

Results are shown in tabular form in Figure 2.
30 Suppression by 24h incubation in human plasma of CSH-induced (880μM x 6 h) glial HO-1 mRNA band (Northern blot) relative to CSH-treated astrocytes grown in standard culture media; 0=0-25% suppression, 1=26-50% suppression, 2=51-75% suppression, 3=76-100% suppression. HOS = HOS activity; MMSE = Folstein Mini-
35 mental State Exam Score; Cortisol = Plasma cortisol levels (nMol/L). AD Meds = cholinesterase inhibitors used for treatment of Alzheimer disease. E400 and E800 = 400 and 800

29

units vitamin E, respectively; C500 = 500 mg vitamin C. HOS
activity was assayed as described in Example 1. Measurement of
cortisol levels were performed using the GammaCoat [I-125]
Cortisol Radioimmunoassay (RIA) Kit based on the competitive
5   binding principles of RIA.

**Example 3: HOS activity in normal control (NC), mild cognitive
impairment (MCI) and sporadic Alzheimer disease (AD) subjects.**

        Results are shown in Figure 3. HOS activity =
percentage suppression (quartiles) by 24h incubation in human
10 plasma of CSH-induced (880μM x 6 h) glial HO-1 mRNA band
(Northern blot) relative to CSH-treated astrocytes grown in
standard culture media. HOS activity was assayed as described
in Example 1.

**Example 4: Plasma cortisol levels (mean ± SD) in normal
15 control (NC), mild cognitive impairment (MCI) and sporadic
Alzheimer disease (AD) subjects.**

        Results are shown in Figure 4. Panel A shows mean (±
SD) plasma cortisol levels of NC, MCI and AD subjects. ( ) =
number of cases per group. Differences between groups are not
20 statistically significant (1-way ANOVA). Correlations between
plasma cortisol levels and HOS activity in the MCI (panel B)
and AD (panel C) groups are not significant (linear regression
analysis). Although glucocorticoids are known suppressors of
the HO-1 gene, these data indicate that elevated cortisol
25 levels are not responsible for HOS activity in the MCI and AD
plasma.

**Example 5: Effects of sample storage time and antioxidant
exposure on plasma HOS activity.**

        Results are shown in Figure 5. HOS activity was
30 assayed as described in Example 1. C = Control (untreated)
astrocyte cultures, CSH = cysteamine-treated astrocyte culture,
AD = Alzheimer, MCI = Mild Cognitive Impairment, NEC = normal
elderly control, N = normal subject on antioxidants. Protease
inhibitors (Complete Protease Inhibitor Cocktail, Cat. #
35 1836153, Roche, Mannheim) were added to all plasma samples

prior to freezing. HOS activity is retained in AD and MCI
plasma samples stored at -85^0 C for up to 15 months. In normal
subjects, low-dose vitamin E (400U/day), a dose of vitamin E ·
commonly taken by AD patients, does not affect the astrocyte

5 HO-1 mRNA response to CSH (N1). In normal individuals, exposure
to multiple, very high-dose antioxidants partially attenuates
the glial HO-1 mRNA response to CSH (N2, N3).

Example 6: Effects of plasma dilution on HOS activity

Plasma HOS activity was assayed via the determination

10 of HO-1 mRNA levels in treated rat astroglia as described in
Example 1. In this case, the effects of plasma dilution were
examined, as documented in Figure 6.

Lane 1: Absence of HO-1 mRNA in unchallenged rat
astrocytes grown in standard culture media. Lane 2:

15 Cysteamine (CSH; 880μM x 6h)induces strong HO-1 mRNA bands in
cultured astroglia grown in standard media. Lane 3: Absence
of HO-1 mRNA bands in unchallenged astrocytes grown in
Alzheimer (AD) plasma (A: patient 1; B: patient 2). Lanes 4-6:
 undiluted AD plasma markedly suppresses the HO-1 mRNA response

20 to CSH in cultured astroglia (intense HOS activity present).
The glial HO-1 mRNA response to CSH progressively increases
(abrogation of HOS activity) with increasing dilutions of AD
plasma using standard media (lanes 7-15).

Panel A and panel B of Figure 6 represent data

25 obtained using plasma obtained from two different AD patients
(A: patient 1; B: patient 2). As noted above and shown again
in Figure 6, untreated rat astroglia grown in standard culture
media exhibit little or no detectable HO-1 mRNA (lane 1),
however, HO-1 expression increases significantly upon CSH

30 treatment (lane 2). In the absence of CSH treatment, rat
astroglia incubated with AD plasma show no detectable HO-1 mRNA
(lane 3), also as noted in Example 1. CSH treatment of rat
astroglia incubated with undiluted AD plasma ("100%") failed to
induce any significant induction of HO-1 expression (lanes

35 4-6), due to the intense HOS activity present in the undiluted
AD plasma. However, the rat astroglial response to CSH

progressively increases (abrogation of HOS activity) with
increasing dilutions of the AD plasma using standard media
(lanes 7-15). Therefore, there appears to exist in AD plasma a
HOS factor whose plasma concentration correlates with HOS
5   activity.

Example 7:   Effect of heat treatment on HOS activity

Plasma HOS activity was assayed via the determination
of HO-1 mRNA levels in treated rat astroglia as described in
Example 1. In this case, the effects of prior heat treatment
10   of AD plasma were examined, as documented in Figure 7.

As noted above, control rat astroglia grown in
standard media or exposed to human plasma (from normal [NEC]
subjects or AD patients) exhibit little or no HO-1 mRNA via
Northern analysis in the absence of CSH treatment (lanes 1, 5,
15   7 and 10). CSH treatment (880 µM CSH x 6h) of astroglia grown
in standard media results in the observation of an intense HO-1
mRNA signal (lane 2). Also as noted above, this induction of
HO-1 expression in response to CSH treatment is significantly
attenuated in astroglia incubated in AD plasma for 24 h (lanes
20   3 and 4). However, this attenuation is no longer observed when
the AD plasma is heated (100°C for 10 min.) prior to incubation
with rat astroglia, indicating that as a result of this pre-
heating AD plasma HOS activity is abrogated, as observed in the
robust HO-1·mRNA signal seen in lane 6. CSH treatment of rat
25   astroglia with normal plasma either untreated or pre-heated
results in a robust observed HO-1 mRNA signal, since HOS
activity is absent in either case (lanes 8 and 9). Therefore,
these data indicate that HOS activity in AD plasma is mediated
by a protein.

30   **Example 8:   Partial purification of HOS factor by heparin-
agarose affinity column chromatography**

Plasma from one normal subject (NEC) and one AD
patient (AD) was subjected to affinity purification on a
heparin-agarose column as described in Sasaki et al. (1987),
35   herein incorporated by reference, as follows:

<u>Plasma preparation for loading onto Heparin Agarose</u>
<u>column</u>: The NEC and AD plasma tubes were thawed at 4°C. The
samples were then dialyzed against Heparin Agarose column
loading buffer [HALB: 20 mM Hepes (SIGMA Chemical Co., Saint

5   Louis, MI, USA, Catl # H-4034) pH 7.2, 150 mM NaCl, protease
inhibitor tablet (Roche Diagnostics, Laval, PQ, CANADA Catl. #
1 873 580)] for 2h with gentle stirring. The samples were then
centrifuged at 15,000 g at 4°C for 20 minutes and supernatants
collected.

10   <u>Heparin Agarose affinity column chromatography</u>: The
Heparin Agarose column (1 cm X 2 cm; SIGMA Chemical Co., Saint
Louis, MI, USA, Catl # H-0402) was prewashed with 20 ml of
HALB. Plasma supernatants were loaded on the column. The
column was washed with 4-6 volumes of HALB and 1 ml fractions

15   collected. The flow-through fractions containing protein were
pooled. The column was eluted with elution buffer [EB: 20 mM
Hepes pH 7.2, 1 M NaCl, protease inhibitor] and 1 ml eluates
containing proteins were pooled and dialyzed against HALB for
2-4h.

20   The preparation of protein (e.g. plasma or column
fractions) for the rat astroglia/HOS activity assay was
performed as described in Sasaki et al. (1987), herein
incorporated by reference, as follows:

           Media was removed from 70 ml, 25 cm^2 flasks containing
25   confluent astrocyte monolayer (7-10 days in culture). To each
individual flask, 1.4 ml of NEC or AD plasma was added. To
each individual flask, approximately 1.4 ml (10 mg protein
based on the Bradford BioRad Protein assay kit using BSA as
control) of the Heparin Agarose flow-through fraction derived

30   from NEC or AD subjects was added with 0.6 ml of complete DMEM
medium. To each individual flask, approximately 1.4 ml (0.5 mg
protein) of the Heparin Agarose eluate fractions derived from
NEC or AD subjects was added with 0.6 ml of complete DMEM
medium.

35   Subsequently, the various samples were assayed for
HOS activity as described in Example 1. Briefly, the eluate

samples were incubated with rat astroglia, which were then
subjected to CSH treatment. Subsequent mRNA isolation and
Northern analysis to determine the level of HO-1 mRNA was
performed, with the results shown in Figure 8.

5   Figure 8: Panel A: Northern blots of HO-1 mRNA. Panel B:
Control GAPDH mRNA. Plasma from one NEC and one AD patient
was affinity purified on a heparin-agarose column and the
eluate, collected using a high salt solution, was dialyzed. In
the absence of CSH treatment, control rat astroglia pre-
10   incubated with heparin eluates from NEC or AD plasma for 24h
did not exhibit an increase in HO-1 expression, as observed by
the relatively faint HO-1 mRNA bands which correspond to these
samples (lanes 1 and 4). CSH treatment (880 μM CSH x 6h) of
rat astroglia incubated with the heparin eluate from NEC plasma
15   results in an induction of HO-1 expression, as observed by
intense HO-1 mRNA bands (lanes 2 and 3). Conversely, no
augmentation of HO-1 mRNA bands in response to CSH treatment
was observed in rat astroglia incubated for 24h with the
heparin eluate fraction derived from the plasma of the AD
20   patient. These data support the presence of a HOS factor in
the plasma of AD patients, but not normal (NEC) subjects, and
indicate that the factor binds to heparin-agarose affinity
columns.

Example 9: Further HOS purification of heparin agarose eluate
25 **by Concanavalin-A (Con-A) Agarose affinity column**
chromatography.

        Heparin Agarose column eluate (as described in Fig.
8) was dialyzed against loading buffer: 50 mM Hepes, pH 7.2
containing 150 mM NaCl, 1 mM $MgCl_2$, 1mM $MnCl_2$, 1 mM $CaCl_2$ and
30   Complete™ EDTA-free Protease inhibitor cocktail for 4 h at 4 °C.
The dialysate was loaded onto Con-A Agarose column. The column
was washed with four bed volumes of loading buffer. The HOS
fraction was eluted with loading buffer containing 0.2M α-D-
methyl mannopyranoside. The eluate was dialyzed against loading
35   buffer. The HOS bioassay (glial HO-1 mRNA response to 880μM CSH
x 6 h) was performed as described in Example 1 and Figure 1.
Results are shown in figure 9. Glial HO-1 mRNA bands were

faint in all specimens not exposed to CSH. Robust HO-1 mRNA
responses to CSH were observed in control astroglial cultures
(grown in standard culture media) and astrocytes incubated for
24 hours in NEC plasma. In contrast, HO-1 mRNA responses to CSH
5   were markedly suppressed in astrocytes incubated for 24 hours
in (i) whole AD plasma, (ii) dialyzed AD plasma prior to
heparin-ConA chromatography and (iii) heparin Agarose-ConA
eluate derived from AD plasma These data indicate that the AD
plasma HOS factor binds to ConA columns and is therefore likely
10 a glycoprotein.

**Example 10:   Further HOS purification of heparin agarose-
conconavalin A eluate derived from 4 pooled AD plasma samples
(29 cc starting material) on Superose™ 12 HR FPLC Column.**

Results are shown in Figures 10 to 12.   Heparin
15 Agarose – ConA Agarose purified AD plasma (1-ml) was dialyzed
against 20 mM Hepes, pH 7.2 containing 150 mM NaCl and
Complete™ EDTA-free Protease inhibitor cocktail (1 tablet/100-
ml; Catl. # 1873580, Lot 61320101; Roche Diagnostics, Quebec,
Canada) for four hours at 4 °C. The dialyzed fraction was loaded
20 on Superose™ 12 HR FPLC 1-cm diameter column (Catl. # 17-0538-
01, Lot # 8283034) [Amersham Pharmacia Biotech, Inc Quebec
Canada]. HOS activity was measured by bioassay in each fraction
as described in Example 1 and Figure 1. As shown in Figure 10,
robust HOS activity was observed in fraction number 20-22.

25   Figure 11: Relative protein concentrations in
Superose™ 12 HR FPLC Column fractions derived from pooled AD
plasma samples described in Fig. 10. Each 0.5-ml fraction of
the flow-through was collected and absorbance (O.D.) measured
at 280 nm by spectrophotometer. A graph of O.D. versus fraction
30 number is plotted. Arrow denotes protein concentration in
fraction (number 20-22) exhibiting robust HOS activity (Figure
11).

Figure 12: A chromatogram from a function test of
Superose™ 12 HR FPLC 1-cm diameter column (Catl. # 17-0538-01,
35 Lot # 8283034) [Amersham Pharmacia Biotech, Inc Quebec Canada]
using standard protein mixtures. Plasma samples were derived

from the same pooled AD plasmas described in Fig. 10. Each 0.5-ml fraction of the flow-through was collected and absorbance measured at 280 nm. A graph of peak molecular weight for each protein standard versus fraction number was plotted. Based on
5   the elution profile of standards with known molecular weight, the molecular weight of the HOS-positive fraction (number 20-22) is estimated at approximately 90 kDa.

Example 11: Effects of NEC and AD plasma on astrocyte HO-1 mRNA induction by multiple stimuli.

10   The HOS bioassay was performed as described in Example 1 and Figure 1. Northern blots for HO-1 mRNA (top) and respective GAPDH mRNA (bottom) are shown in Figure 13. AD plasma strongly suppressed the HO-1 mRNA response to CSH (880μM), interleukin-1β (Il-1β 50 and 100 ng/ml), and
15 homocysteine (HC 200μM). NEC plasma showed no HOS activity in the face of these stimuli. AD plasma completely suppressed, whereas NEC plasma partially suppressed, the HO-1 mRNA response to tumour necrosis factor-α (TNF-α 50 and 100 ng/ml). AD and NEC plasma exhibited partial and equal suppression of the glial
20 HO-1 mRNA response to menadione (Mena 100μM). These data indicate that: A) The HOS protein in AD plasma is active against multiple inducers of astroglial HO-1 mRNA. B) HOS activity in AD plasma is particularly potent in the face of TNF-α challenge. Since partial HOS activity against TNF-α also
25 occurs in NEC plasma, differences in HOS protein expression between AD and NEC may be quantitative rather than qualitative. C) Simple antioxidant behaviour does not account for HOS activity in AD plasma because both AD and NEC plasma exhibit partial and equal suppression of the glial HO-1 mRNA response
30 to the pro-oxidant, menadione.

      The above examples illustrate application of the testing method to detect HOS activity. Further, the above examples illustrate the testing method using plasma as the tissue or body fluid sample. The method can also be applied to
35 other tissue or body fluids such as blood, cerebrospinal fluid,

urine, saliva, epithelia and to fibroblast cell lines derived
from patients.

The test can be applied to compare the level of HOS
activity in a specific patient over a period of time, or to
5   compare the level of HOS activity in a patient with the
corresponding level in a normal control population.

The above examples further demonstrate the presence of a
HOS factor which is a glycoprotein, in an embodiment a
mannoprotein, having an approximate molecular weight in the
10   range of 80-100 kDa, in an embodiment having a molecular weight
of approximately 90 kDa, and which is not a glucocorticoid.

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CLAIMS:

1.   A heme oxygenase-1 suppressor (HOS) factor, wherein said factor attenuates the increase in the level of heme oxygenase-1 (HO-1).

5   2.   The factor of claim 1, wherein said attenuation occurs in response to exposure to an experimental agent or treatment which is capable of increasing the level of HO-1.

3.   The factor of claim 1, wherein the factor comprises a protein.

10 4.   The factor of claim 2, wherein the exposure to an experimental agent or treatment comprises exposure to any one or more of oxidative stress, metal ions, amino acid analogues, sulfhydryl agents, interleukin-1β, tumour necrosis factor-α (TNF-α) and hyperthermia.

15 5.   The factor of claim 4, wherein the sulfhydryl agent is selected from the group consisting of cysteamine and homocysteine.

6.   The factor of claim 5, wherein the sulfhydryl agent is cysteamine.

20 7.   A method for assessing dementing diseases in a patient which comprises:

    determining the level of heme oxygenase-1 suppressor (HOS) factor or activity, in tissue or a body fluid obtained from a patient; and

25   comparing said level of HOS factor or activity with the corresponding level of HOS factor or activity in corresponding tissue or body fluid obtained from at least one control person, whereby if said level of HOS factor or activity is greater than said corresponding level of HOS factor or

30 activity in said tissue or body fluid obtained from at least one control person then said patient suffers from a dementing disease;

wherein such method is used to predict the onset of, diagnose, or prognosticate dementing diseases.

8.   A diagnostic method for differentiating, in a patient suffering from a dementing disease, between a dementing disease which is HO-1-dependent and a dementing disease which is HO-1-independent, said method comprising:

determining the level of heme oxygenase-1 suppressor (HOS) factor or activity, in tissue or a body fluid obtained from a patient suffering from a dementing disease; and

comparing said level of HOS factor or activity with the corresponding level of HOS factor or activity in corresponding tissue or body fluid obtained from at least one control person,

whereby if said level of HOS factor or activity differs significantly from said corresponding level of HOS factor or activity in said tissue or body fluid obtained from at least one control person then said patient suffers from a dementing disease which is HO-1-dependent, and if said level of HOS factor or activity does not differ significantly from said corresponding level of HOS factor or activity in said tissue or body fluid obtained from at least one control person then said patient suffers from a dementing disease which is HO-1-independent.

9.   The method according to claim 7 wherein the tissue or body fluid is selected from blood, plasma, lymphocytes, cerebrospinal fluid, urine, saliva, epithelia and fibroblasts.

10.   The method according to claim 8 wherein the tissue or body fluid is selected from blood, plasma, lymphocytes, cerebrospinal fluid, urine, saliva, epithelia and fibroblasts.

11.   The method according to claim 7 wherein the dementing
disease is selected from the group consisting of Alzheimer
Disease, Age-Associated Cognitive Decline, Mild Cognitive
Impairment, Parkinson disease with dementia, Progressive
5 Supranuclear Palsy, Vascular (i.e. multi-infarct) Dementia,
Lewy Body Dementia, Huntington's Disease, Down's syndrome,
normal pressure hydrocephalus, corticobasal ganglionic
degeneration, multisystem atrophy, head trauma, neurosyphilis,
Creutzfeld-Jacob disease and other prion diseases, HIV and
10 other encephalitides, and metabolic disorders such as
hypothyroidism and vitamin B12 deficiency.

12.   The method of claim 7 wherein the control person is a
normal age-matched person.

13.   The method of claim 7 wherein the method is used to
15 prognosticate dementing diseases and wherein the control person
is the patient from whom the corresponding tissue or body fluid
was obtained at another time.

14.   The method according to claim 8 wherein the HO-1-
dependent dementing disease is Alzheimer Disease.

20 15.   The method of claim 8 wherein the control person is a
normal age-matched person.

16.   The method of claim 8 wherein the control person is
the patient from whom the corresponding tissue or body fluid
was obtained at another time.

25 17.   A method for assaying the level of heme oxygenase-1
(HO-1) suppressor (HOS) factor or activity in a sample which
comprises:

exposing the sample to a cell culture;

subjecting the cell culture to exposure to an
30 experimental agent or treatment which may increase the level of
HO-1 protein or mRNA encoding HO-1;

determining the level of HO-1 protein or mRNA
encoding HO-1; and

comparing said level of HO-1 protein or mRNA encoding HO-1 with a corresponding control level of HO-1 protein or mRNA encoding HO-1;

whereby the level of said HO-1 protein or mRNA
5   encoding HO-1 inversely correlates with the level of HOS factor or activity.

18.   A method for assaying the level of heme oxygenase-1 (HO-1) suppressor (HOS) factor or activity in a sample comprising:

10   exposing said sample to an antibody which recognizes the factor of claim 1;

isolating immune complexes; and

determining the level of HOS factor or activity in the immune complex.

15   19.   A method for assaying the level of heme oxygenase-1 (HO-1) suppressor (HOS) factor or activity in a sample comprising:

exposing said sample to a reporter construct, wherein said reporter construct comprises the HO-1 promoter region and
20   a reporter gene, wherein said reporter gene encodes a protein which possesses a detectable reporter activity;

determining the level of said reporter activity; and

comparing said level of said reporter activity with a corresponding control level of said reporter activity;

25   whereby the level of said reporter activity inversely correlates with the level of HOS factor or activity.

20.   A method for screening a candidate compound for the presence of an inhibitor or activator of HOS activity or HOS factor comprising:

30   exposing said candidate compound to a sample known to comprise HOS activity or HOS factor;

assaying the level of HOS activity or HOS factor
using a method selected from the group consisting of:

(a) a method for assaying the level of heme
oxygenase-1 (HO-1) suppressor (HOS) factor or activity in a
5 sample which comprises:

exposing the sample to a cell culture;

subjecting the cell culture to exposure to an
experimental agent or treatment which may increase the level of
mRNA encoding HO-1;

10 determining the level of HO-1 protein or mRNA
encoding HO-1; and

comparing said level of HO-1 protein or mRNA encoding
HO-1 with a corresponding control level of HO-1 protein or mRNA
encoding HO-1;

15 whereby the level of said HO-1 protein or mRNA
encoding HO-1 inversely correlates with the level of HOS factor
or activity;

(b) a method for assaying the level of heme
oxygenase-1 (HO-1) suppressor (HOS) factor or activity in a
20 sample comprising:

exposing said sample to an antibody which recognizes
the factor of claim 1;

isolating immune complexes; and

determining the level of HOS factor or activity in
25 the immune complex; and

(c) a method for assaying the level of heme
oxygenase-1 (HO-1) suppressor (HOS) factor or activity in a
sample comprising:

exposing said sample to a reporter construct, wherein
30 said reporter construct comprises the HO-1 promoter region and

a reporter gene, wherein said reporter gene encodes a protein
which possesses a detectable reporter activity; and

determining the level of said reporter activity; and

comparing said level of said reporter activity with a
5   corresponding control level of said reporter activity;

whereby the level of said reporter activity inversely
correlates with the level of HOS factor or activity;

and comparing said level of HOS activity or HOS
factor with a corresponding control level of HOS activity or
10 HOS factor in a corresponding control sample, wherein said
control sample comprises said sample known to comprise HOS
activity that has not been exposed to said candidate compound.

21.   A commercial package comprising means for determining
the level of heme oxygenase-1 (HO-1) suppressor (HOS) activity,
15 in tissue or body fluid obtained from a patient, and
instructions for comparing said level of HOS activity with an
established standard of the corresponding HOS activity in
corresponding control tissue or body fluid.

22.   The commercial package of claim 21 for use in a
20 method for assessing dementing diseases in a patient.

23.   A method of treating a dementing disease in a
patient, comprising administering to said patient a compound
which alleviates the dementing disease by increasing or
decreasing the level of heme oxygenase-1 (HO-1) mRNA, protein
25 or activity, in an amount effective to treat a dementing
disease, wherein said method results in the alleviation of the
dementing disease by increasing or decreasing the level of any
one or heme oxygenase-1 (HO-1) mRNA, HO-1 protein , HO-1
activity, HO-1 suppressor (HOS) factor, and HOS activity.

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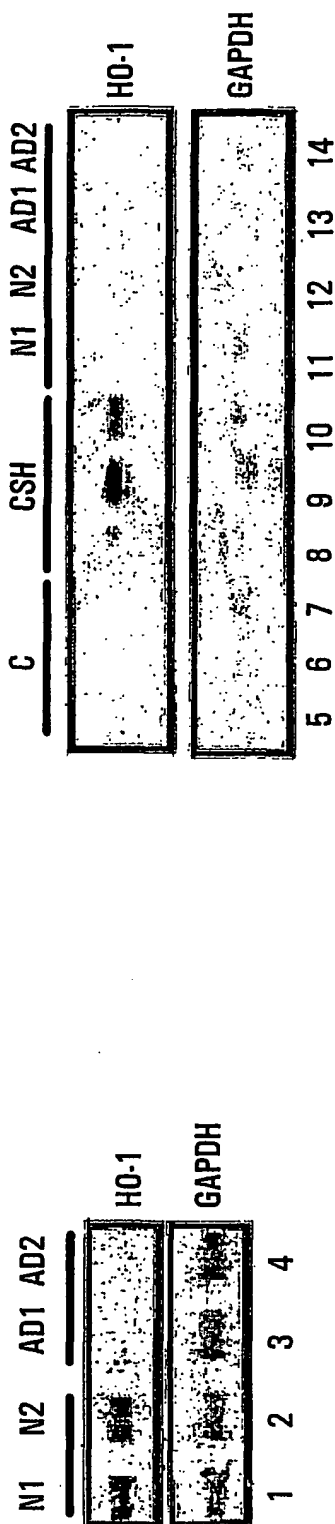
FIG. 1A
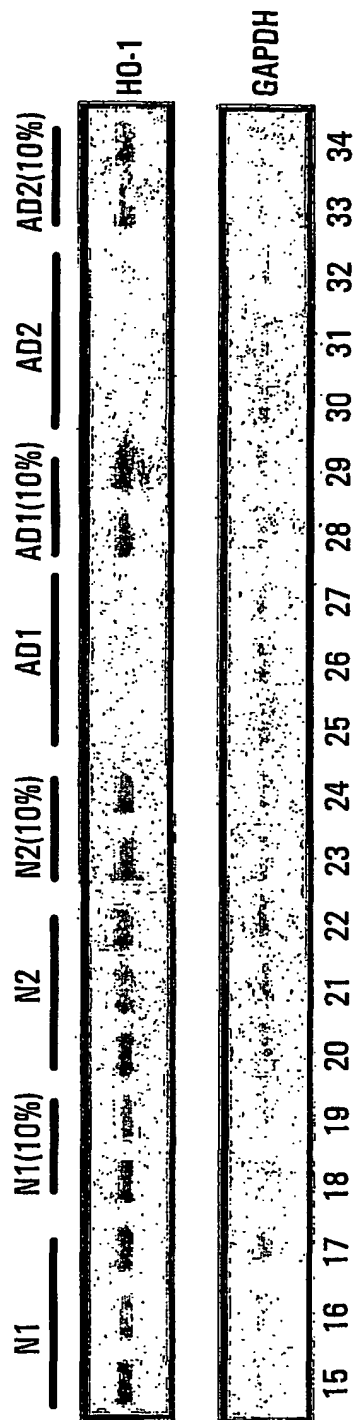
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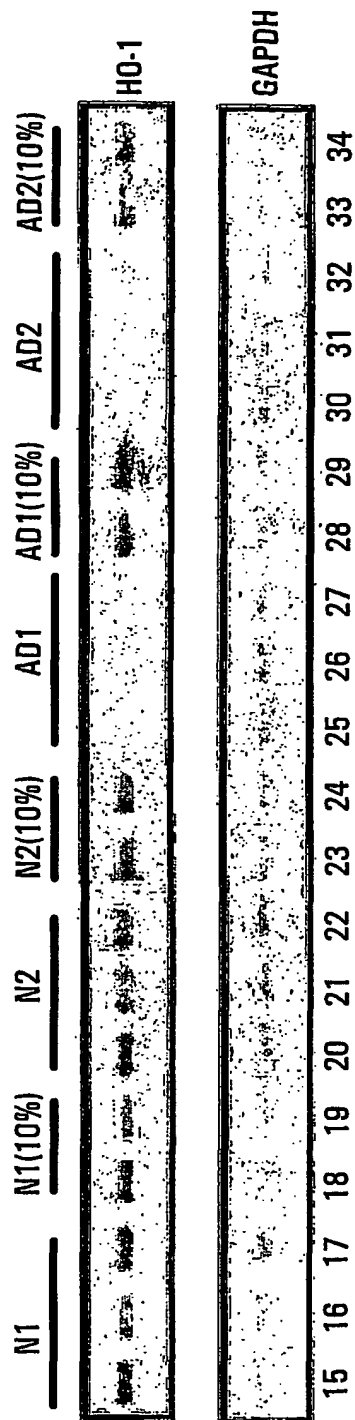
FIG. 1C

PATIENT	DIAGNOSIS	HOS	AGE	MMSE	SEX	AD MEDS	ANTIOX.	CORTISOL
295	NYC	0	42	30	F	NONE	NONE	215
296	NYC	0	56	28	F	NONE	NONE	163
297	NYC	0	25	30	M	NONE	NONE	234
298	NYC	0	35	30	F	NONE	NONE	183
299	NYC	0	40	30	F	NONE	NONE	121
300	NYC	0	47	30	F	NONE	NONE	245
301	NYC	0	39	30	F	NONE	NONE	
302	NYC	0	29	30	F	NONE	NONE	
319	NEC	0	67	28	M	NONE	NONE	176
325	NEC	0	66	29	F	NONE	NONE	
333	NEC	0	89	29	M	NONE	NONE	129
345	NEC	0	71	28	F	NONE	NONE	225
346	NEC	0	83	29	M	NONE	NONE	163
731	NEC	0	92	29	F	NONE	NONE	
335	MCI	2	79	29	M	NONE	NONE	270
337	MCI	0	56	30	M	NONE	NONE	
344	MCI	2	80	28	M	NONE	NONE	
351	MCI	0	79	28	M	NONE	E800,C500	259
352	MCI	0	71	25	F	NONE	NONE	
354	MCI	0	72	29	M	NONE	NONE	
358	MCI	0	79	28	M	NONE	E400,C500	289
472	MCI	3	82	27	F	NONE	NONE	312
293	AD	3	66	13	F	ARICEPT	NONE	
304	AD	3	70	26	M	ARICEPT	NONE	
305	AD	3	60	17	M	ARICEPT	NONE	
306	AD	3	83	19	F	NONE	NONE	
307	AD	3	68	21	F	NONE	NONE	292
310	AD	3	55	19	F	ARICEPT	NONE	136
312	AD	3	81	28	M	NONE	NONE	285
316	AD	3	78	28	M	ARICEPT	NONE	184
322	AD	2	68	13	F	ARICEPT	NONE	
328	AD	2	77	25	M	ARICEPT	NONE	69
330	AD	3	77	21	M	NONE	NONE	407
336	AD	3	81	23	M	NONE	NONE	278
342	AD	1	84	27	F	NONE	NONE	
343	AD	3	87	20	F	ARICEPT	NONE	187
347	AD	1	71	19	F	ARICEPT	NONE	265
355	AD	3	76	24	M	ARICEPT	E400	135
356	AD	3	83	23	F	NONE	NONE	274
359	AD	3	66	21	F	ARICEPT	E800	325
360	AD	3	82	20	M	NONE	NONE	311
475	AD	3	62	29	M	ARICEPT	C500	292
714	AD	2	70	24	F	NONE	NONE	
730	AD	2	81	24	M	NONE	NONE	
294	AD	3	80	25	M	ARICEPT	NONE	

FIG. 2
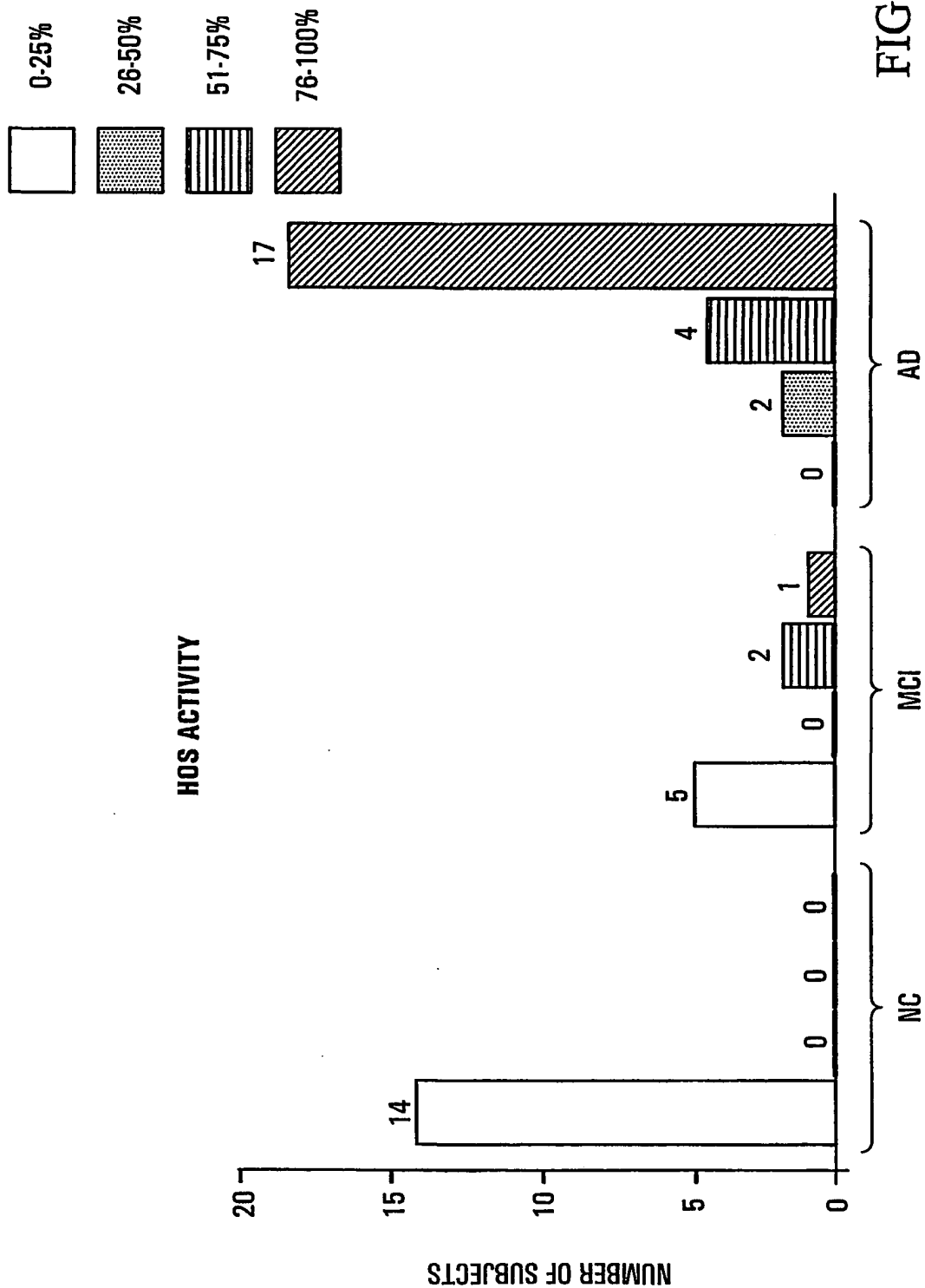
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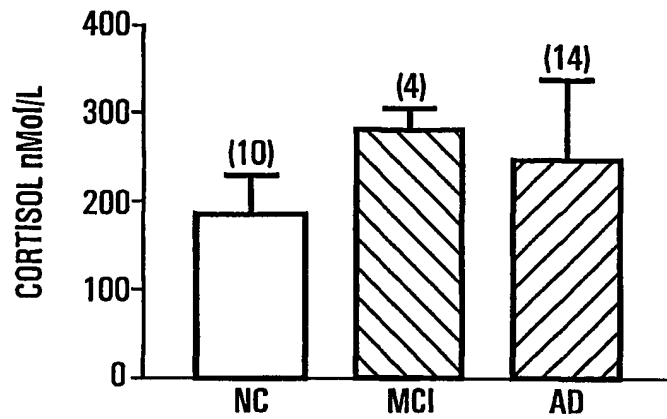
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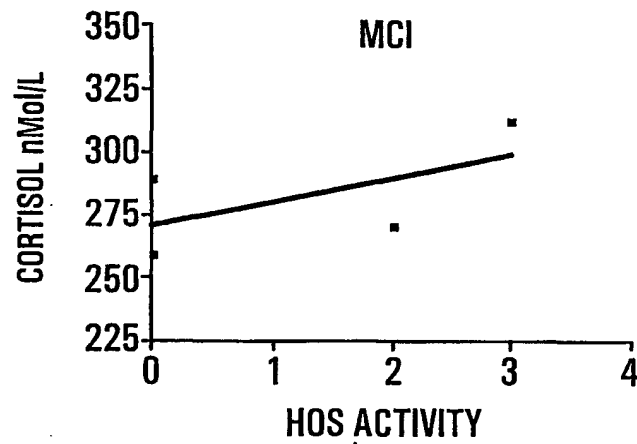
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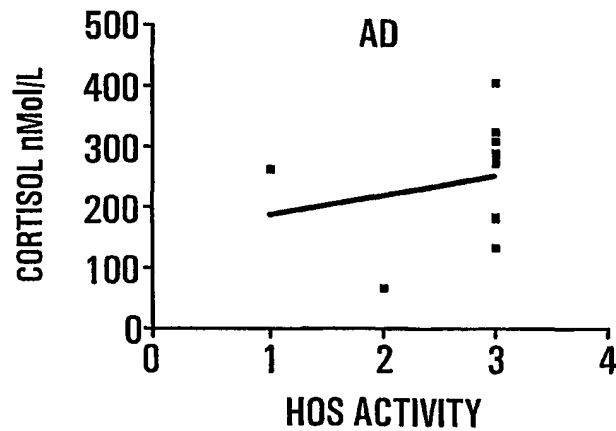
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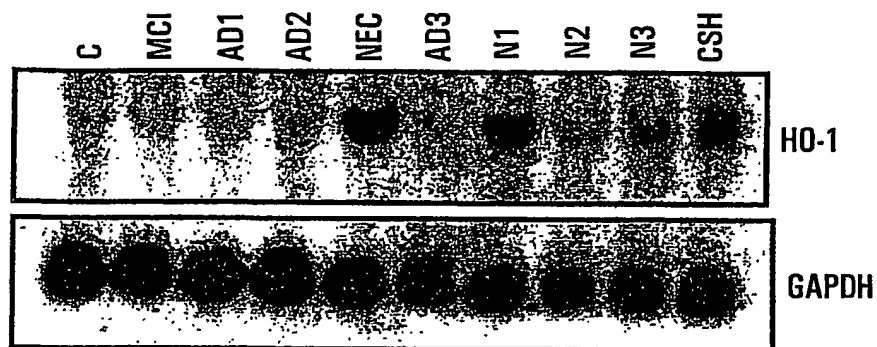
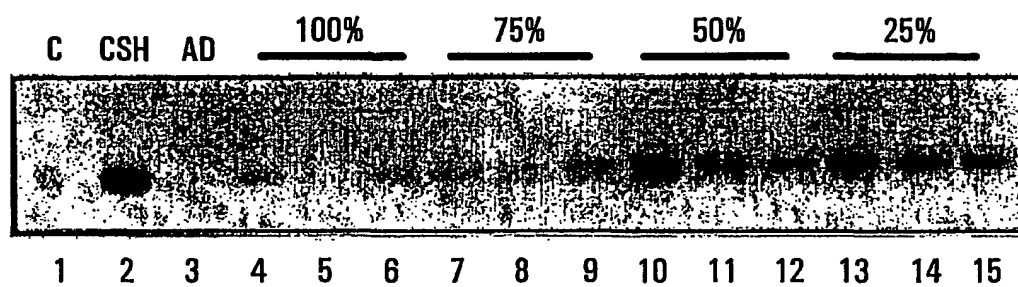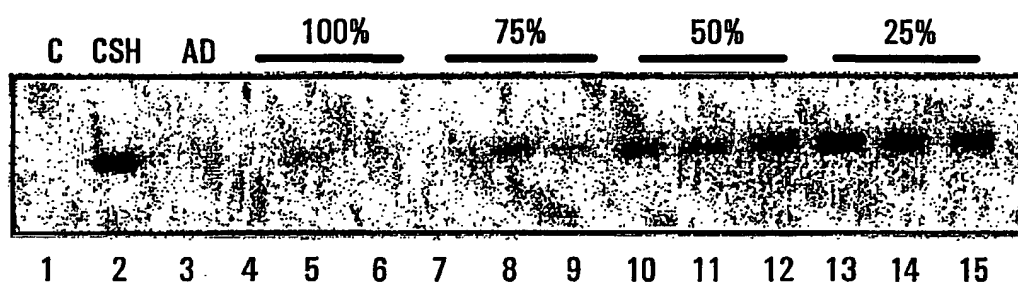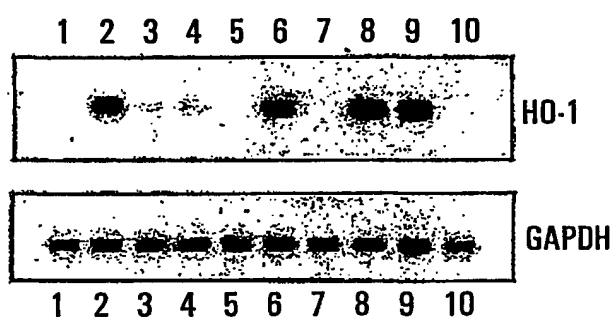| SAMPLE	STORAGE TIME	ANTI-OXIDANT
MCI	15 MONTHS	NONE
AD1	15 MONTHS	VIT C 500 mg
AD2	2 MONTHS	NONE
NEC	9 DAYS	NONE
AD3	9 DAYS	NONE
N1	9 DAYS	VIT E 400U
N2	9 DAYS	VIT C 2000mg, COENZYME Q10 100mg, E 400U
N3	9 DAYS	α LIPOIC ACID 600mg, LYCOPENE 15mg BETA CAROTENE 10,000U, VIT C 1000mg, E 400U

FIG. 5

FIG. 6A

FIG. 6B

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FIG. 7
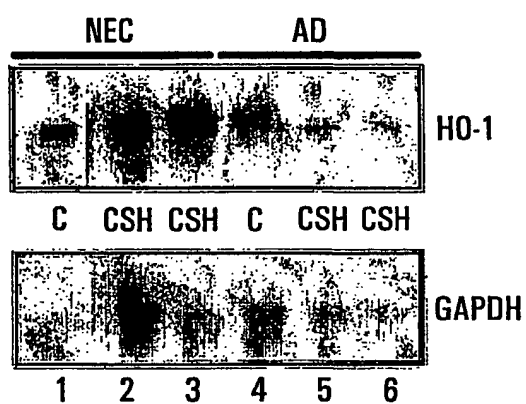
FIG. 8

HOS HepA- ConA PURIFICATION

FIG. 9

FIG. 10

FIG. 11

FIG. 12

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FIG. 13